CHARACTERIZATION OF A WILD POLIOVIRUS TYPE 3 ISOLATED JAPAN IN 1993

Tetsuo YONEYAMA, Takashi FUJIWARA*, Yoko YOKOTA1, Yoshimi TAKEMIKA1 and Akio HAGIWARA

Laboratory of Enteroviruses, Department of Virology II, National Institute of Health, 4-7-1 Gakuen, Musashimurayama-shi, Tokyo 208, and 1the Shiga Prefectural Institute of Public Health and Environmental Science, 13-45 Gotenhama, Ohtsu-shi, Shiga 520

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SUMMARY: A wild poliovirus type 3 was isolated from a throat swab of a patient with upper respiratory symptoms without paralysis in Shiga Prefecture, in 1993. Wild poliovirus has never been isolated in these nine years in Japan. The most recent isolation of wild poliovirus was of type 1 in 1984 from a case of encephalomyelitis in Aichi Prefecture. Antigenic and PCR-restriction fragment length polymorphism analyses revealed that the Shiga strain was non-Sabin origin. Sequence analysis of the VP1 region confirmed that the isolate was a wild poliovirus type 3. Furthermore, this isolate had higher homology to the isolates from North Vietnam than those from Pakistan or Finland, suggesting that the Shiga strain was imported from Asian area. This strain was also shown to be neurovirulent in transgenic mice carrying human poliovirus receptor gene.

INTRODUCTION

After mass immunization with oral polio vaccine (OPV) in 1961 in Japan, poliomyelitis caused by wild polioviruses is considered to has been completely
controlled except for three reported cases due to type 1 in 1968 and 1980 and type 3 in 1971 (1,2). In addition to the above three cases, type 1 wild polioviruses were isolated from a patient with encephalomyelitis in 1984 (3), and from wastes of a jet-airliner at Narita airport in 1981 (4). In either case, however, the virus isolation was not associated with paralytic poliomyelitis. Poliomyelitis is still endemic in many developing countries in Asia, although the incidence has decreased dramatically (5). The more active the international transportation, the more often the importation of wild polioviruses. In this paper, we report isolation of a neurovirulent wild poliovirus type 3 from a feverish patient with upper respiratory symptoms but with no typical symptoms of poliomyelitis. By nucleotide sequence analysis, the virus was suggested as a wild type strain from an Asian area.

MATERIALS AND METHODS

Report of a case: A 13-year-old boy visited a hospital because of his high fever (39.2°C) and upper respiratory symptoms on January 19, 1993. Three days later, a throat swab was taken. The disease was transient and left no residual damages. No neurological abnormality was observed during the course of the disease. He had one OPV at one year and 4 months old. Incidentally, he had a short trip to Taiwan from December 28, 1992 to January 1, 1993 before the onset of the disease (6).

Virus isolation and identification: Poliovirus was isolated from the throat swab of the patient in cell cultures of HEp-2 and RD at 36°C. Poliovirus antiserum pools were used for identification by the standard microplate neutralization test.

Intratypic differentiation methods: [1] Antigenic analysis was carried out by the neutralization test with monoclonal antibodies, 92/546 (Sabin 3 specific) and 92/548 (poliovirus type 3 specific), provided by Dr. Minor (National Institute for Biological Standards and Control, UK) through WHO. [2] PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis was done by the method of Balanant et al (7). In brief, the 474-nucleotide sequence of type 3 poliovirus genome coding the N-terminal half of the capsid protein VP1 (nucleotide 2399-2872) was targeted and amplified from purified viral RNA with a set of primers, UC-1, 5'-GAATTCCATGTCAAATCTAGA and UG-1, 5'-TTTGTGTCAGCGTAATGA. Another target region, the 431 nucleotide segment in the 3D polymerase-coding region (nucleotide 6077-6507), was amplified with a set of primers, UC-12, 5'-TCAATTAGTCTGGATTTCCCTG
and UG-7, 5'-TTTAAGGGGTTAGGAACCAGC (8). Nucleotide positions were numbered according to Sanway et al (9).

**Sequence analysis:** After purification with glassmilk (GENECLEAN II Kit, BIO 101 Inc., USA), the PCR-product of the VP1 region was directly sequenced with a dye-terminator cycle sequencing kit (Applied Biosystems) with an Automatic sequencer ABI 373A 18. The primers, UC1 and UG1, the same ones used in PCR-RFLP assay, were also employed.

**Neurovirulence test:** A transgenic mouse line, ICR-PVRTg 21 (Tg21), which is susceptible to poliovirus was used to test the isolate for the neurovirulence (10). Five to 6 weeks old Tg21 mice were used in this study. Into the brain of each Tg21 mouse, 30 µl of each of serial dilutions of a virus suspension was inoculated. Five mice were used for each dilution. The number of mice showing paralysis and death within 14 days after the inoculation were scored as paralysis. The 50% paralytic dose (PD50) was calculated by the Kärber method (11).

**RESULTS**

**Intratypic Serodifferentiation**

The isolate (93-Shiga) was identified as poliovirus type 3 with an antipolio serum pool. Antigenic difference between 93-Shiga and Sabin type 3 was investigated by the neutralization test with monoclonal antibodies. Sabin 3 specific antibody, 92/546, could not neutralize 93-Shiga, while type 3-specific antibody, 92/548, neutralized this strain. The 93-Shiga strain was non-vaccine-like poliovirus (Table I). The same serological result was obtained by the McBride and Wecker methods (2) (data not shown).

**PCR-RFLP Analysis**

The electropholetic profiles of 93-Shiga in PCR-RFLP in the VP1 region were different from those of Sabin 3 as shown in Fig. 1. The result of PCR-RFLP in the VP1 region was consistent with those results obtained by the intratypic serodifferentiation methods described above. PCR-RFLP of the 3D polymerase region was also carried out after amplifying 431 nucleotides. The profiles of 93-Shiga strain were also different from those of Sabin 3 in the 3D region. The result indicated the sequence of 93-Shiga in this region was also non-Sabin like (Fig. 2).
Table I. Intratypic serodifferentiation by monoclonal antibodies

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Neutralization titer with</th>
<th>Antigenic character</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>92/546*</td>
<td>92/548**</td>
</tr>
<tr>
<td>Sabin3</td>
<td>&gt;2,560</td>
<td>&gt;2,560</td>
</tr>
<tr>
<td>Saukett</td>
<td>&lt;20</td>
<td>&gt;2,560</td>
</tr>
<tr>
<td>93-Shiga</td>
<td>&lt;20</td>
<td>&gt;2,560</td>
</tr>
</tbody>
</table>

* Sabin3-specific monoclonal antibody.
** Polio3-specific monoclonal antibody.

Fig. 1. PCR-RFLP patterns of 93-Shiga and Sabin 3 vaccine strains. The viral RNA was extracted, reverse-transcribed, and amplified by the PCR method. The target was 474 nucleotides of the N-terminal region of the VP1 of poliovirus. The PCR product was digested with Dde I, Hpa II, or Hae III. Digested products were run on a 3% agarose gel in parallel with molecular-weight markers (φX174/Hae III) and an uncut (UC) cDNA fragment derived from Sabin 3 strain.
Fig. 2. PCR-RFLP patterns of 93-Shiga and Sabin 1, 2, and 3 vaccine strains. The target region included 431 nucleotides in the 3D polymerase region of poliovirus. The PCR product was digested with Dde I, Hae III, or Rsa I. Digested products were analyzed as described in Fig. 1.

Nucleotide Sequence Analysis in VP-1 Region

To investigate the origin of 93-Shiga strain, several polio type 3 isolates were sequenced directly after PCR-amplification. Sequence information on 300 nucleotides (nucleotides numbers 2476 to 2777 corresponding with those of the 5'-terminal part of VP1) from 93-Shiga strain was compared to other polio type 3 isolates including Sabin 3 vaccine strain for similarity (Fig. 3). Over the sequence of 300 nucleotides, 93-Shiga strain was different from Sabin 3 by 79 nucleotides, while usually Sabin-derived strains differed from Sabin 3 only by a few nucleotides in this region (data not shown). This 93-Shiga strain was different from other isolates by 76 to 79 nucleotides but from Vietnam-isolates by 36 to 39 nucleotides. By pairwise alignment and statistical comparison of nucleotide differences, a dendrogram of virus relationships was generated by the UPMGA computer program (GENETYX, Software Developing Co., Ltd., Japan). The 93-Shiga strain was more closer to two Vietnam isolates in 1992 than other isolates from Pakistan in 1991, Finland in 1984, Japan in 1956 and Sabin 3 (Fig. 4).
Fig. 3. Comparison of nucleotide sequences at VP1 region of type 3 poliovirus isolates. Nucleotides different from 93-Shiga are shown; dots indicate identical nucleotides. Nucleotide positions are numbered according to Stanway et al (9). The 300 nucleotides shown constitute the total sequence information used for each strain to construct the dendrogram in Fig. 4. NV92-5 and NV92-6 were isolated from paralytic cases in North Vietnam in 1992. K49 and K60 were isolated from paralytic cases in Karachi, Pakistan in 1991 and 1992, respectively, Z-1 was isolated from a paralytic case in Osaka, Japan in 1956. Suwa was isolated from a healthy child in Nagano, Japan, in 1957. The sequence of 23127 isolated in Finland in 1984 (16) was cited from the EMBL data base.

Table II. Neurovirulence test in transgenic mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>PD50 (Log10 TCID50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabin3</td>
<td>&gt;7.8</td>
</tr>
<tr>
<td>93-Shiga</td>
<td>4.6</td>
</tr>
<tr>
<td>Saukett</td>
<td>6.2</td>
</tr>
</tbody>
</table>
Fig. 4. Dendrogram of polio type 3 strains isolated in different geographic areas. The nucleotide sequence divergence between any two strains is twice the distance along abscissa to the connecting node.

**Neurovirulence Test in Tg-mice**

In the neurovirulence test monkeys can be replaced by Tg-mice for epidemiological studies (12,13). The results of neurovirulence tests in Tg21 mice indicated 93-Shiga strain was virulent strain in Tg-mice in contrast to attenuated Sabin 3 strain (Table II).

**DISCUSSION**

Since the mass administration of OPV in 1961, the incidence of paralytic poliomyelitis has decreased markedly in Japan. However, a few paralytic poliomyelitis cases have still been reported in recent years (1). Usually these cases have occurred either among vaccinees or their contacts soon after the vaccination. During 1981-1992, an average of 2,000 fecal specimens per year were collected from healthy children to examine. According to the survey of fecal specimens for viruses, the frequency of poliovirus isolation was 0.05-0.15% every
year. More than 80 strains of poliovirus isolated from healthy children and clinical cases have been characterized. Most polio isolates have been classified as vaccine-like by serological methods (data not shown). This indicates that wild poliovirus has been eliminated from the Japanese community. The last wild isolate was of type 1 in Aichi Prefecture in 1984 from a case of non-polio-myelitis (3) and of type 3 in Akita Prefecture in 1971 from a case of paralytic polio-myelitis (1). It is still possible, however, that wild strains of poliovirus are being introduced into Japan. In fact, neurovirulent wild polioviruses were isolated from the wastes of a jet airliner arriving at Tokyo via Karachi, Bangkok and Manila (4). It also became clear that the epidemic strains in the Netherlands in 1992 to 1993 had been originated from Indian subcontinent (14). Even polio-free countries have been faced to risks of importation of wild strains. The 93-Shiga strain and North Vietnam strains belong to the same genotypic group suggesting that Shiga strain was imported from some Asian country around Vietnam. Incidentally, the patient from which 93-Shiga was isolated had had a short travel to Taiwan before viral isolation. Since, Taiwan is free from wild polio-myelitis since 1983 (personal communication, Dr. Lin C.-C.) after the outbreak in 1982 (15), it is unlikely that the virus was from Taiwan. The route of transmission of this virus is totally unknown. No polioviruses were detected in Shiga Prefecture during the first half of the year of 1993 after the isolation of the wild strain. While the level of serum neutralizing antibody against poliovirus type 3 of this patient was high, a low level of or no antibody was detected with his parents showing no symptom of poliovirus infection (data not shown). There was no evidence indicating that this strain spread in Japan. The patient received only one dose of OPV in his infant. Incomplete vaccination could not keep antibody level high enough to protect infection of wild poliovirus. The recent epidemic in the Netherlands was caused also by low level of or no immunity in a community with a special religion (14). It is necessary to receive complete vaccination until the day when wild polioviruses have been eradicated from the world and to keep surveillance for invading wild polioviruses.

The etiological agent causing the upper respiratory disease of this patient was unclear, but it is clear that 93-Shiga strain was virulent from the assay by the neurovirulence test in Tg-mice. Unknown host factors may have been involved in the onset of paralysis in humans. The development of gene technology opened a convenient assay for neurovirulence, i.e. human poliovirus-receptor-expressing mice became available as experimental animals instead of
monkeys. This study clearly showed that Tg-mice is useful for biological characterization of isolated polioviruses.

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REFERENCES


